

The 3'-5' ero plus F \rightarrow Y Mut. Project N. _____
Book No. _____

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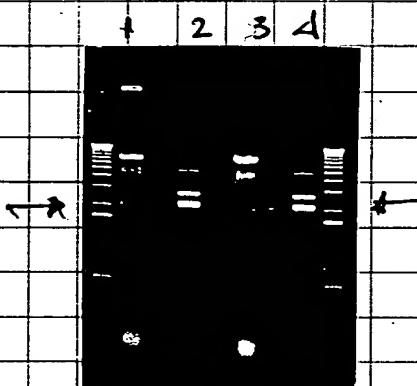
Line

Replace mutant fragment from RF to pUC19-Tne.

I can not do it directly, because there are two Sph I sites in pUC19-Tne.

Therefore, first replace the mutant fragment in pUC19-Tne and then transfer into pUC19-Tne.

Digest pUC19-Tne with Sph I + Hind III. Digest M13mp19 mutants with Sph I + Hind III.



1 = RF 3'-5' ero - Sph I / Hind III
2 = pUC19-Tne "
3 = RF F \rightarrow Y mutant "
4 = pUC19-Tne "

Purify only mutant fragment — to be ligated into pUC19-Tne Sph I / Hind III frag.



5 & 6 pUC19-Tne Sph I / Hind III

Purify the largest fragment. Ligate with the mutant fragments.

Transform DH5 α B.

Plate 10% & 90% →

Overshoot at 30°

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ssed & Understood by me,

Date

Invented by

Date

H. L. J.

6/12/95

Recorded by

Deb A. Kothiyal

5/1/95